Polycyclic Aromatic Hydrocarbons (PAHs), Nitro-PAHs and Related Environmental Compounds: Biological Markers of Exposure and Effects

Glenn Talaska,[1] Patricia Underwood,[1] Andrew Maier,[1] Joellen Lewtas,[2] Nathaniel Rothman,[3] and Marlene Jaeger[1]

[1]Department of Environmental Health, University of Cincinnati Medical School, Cincinnati, Ohio; [2]U.S. Environmental Protection Agency, Research Triangle Park, North Carolina; [3]National Cancer Institute, Bethesda, Maryland

Lung cancer caused by polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs and related environmental agents is a major problem in industrialized nations. The high case-fatality rate of the disease, even with the best supportive treatment, underscores the importance of primary lung cancer prevention. Development of biomarkers of exposure and effects to PAHs and related compounds is now underway and includes measurement of urinary metabolites of specific PAHs as well as detection of protein and DNA adducts as indicators of effective dose. Validation of these markers in terms of total environmental dose requires that concurrent measures of air levels and potential dermal exposure be made. In addition, the interrelationships between PAH biomarkers must be determined, particularly when levels of the marker in surrogate molecules (e.g., protein) or markers from surrogate tissues (e.g., lymphocyte DNA) are used to assess the risk to the target organ, the lung. Two approaches to biomarker studies will be reviewed in this article: the progress made using blood lymphocytes as surrogates for lung tissues and the progress made developing noninvasive markers of carcinogen–DNA adduct levels in lung-derived cells available in bronchial–alveolar lavage and in sputum. Data are presented from studies in which exfoliated urothelial cells were used as a surrogate tissue to assess exposure to human urinary bladder carcinogens in occupational groups. — Environ Health Perspect 104(Suppl 5):901–906 (1996)

Key words: polycyclic aromatic hydrocarbons, lung cancer, biomarkers, DNA adducts, lymphocytes, urothelial cells

Introduction

Lung cancer remains one of the most serious and troubling environmental diseases. The overlapping causes and pathogenesis of the disease make it difficult to estimate the proportion of lung cancer cases attributable to the ambient environment versus those caused by specific agents such as tobacco smoking. However, epidemiological evidence suggests that several occupational groups are at significantly increased risk for developing lung cancer, including populations exposed to polycyclic aromatic hydrocarbons (PAHs) such as coke oven workers and steelworkers (*1*). Table 1 gives a list of the agents identified or strongly suspected of being occupational lung carcinogens, Table 2 gives a list of occupational groups with potential exposure to coal tar, and Table 3 lists complex mixtures containing PAHs. Clearly, there are numerous people in the general population as well as in specific occupational groups who might be exposed to these agents. The focus of this article is the advances that have been made in developing biomarkers of exposure and effect for PAH lung carcinogens and how these markers can provide a framework relevant to the study and prevention of occupational and environmental disease.

Lung cancer is an important disease in the United States and around the world. There were 885,000 cases and 770,000 deaths from lung cancer worldwide in 1985, the last year for which complete statistics are available (*2*). The lung is the site of 11.9% of all tumors worldwide, making lung cancer the most common neoplastic disease in the world. In the United States, lung cancer accounts for 28% of all cancers. There were 170,000 cases of lung cancer in the United States in 1993 and 149,000 deaths (*3*). There is virtually no difference in the 5-year survival rate in the United States and the rest of the world; 87% of lung cancer cases are fatal within 5 years. Thus, an educated public and the availability of modern medical treatment to provide early diagnosis and treatment have had virtually no impact on the course of this disease.

It has been estimated that 76% of all U.S. lung cancer cases are due to tobacco smoking (*4*). The trends for tobacco sales and lung cancer rates are positively correlated within a 20- to 25-year latency period (*3*). Cigarette sales to American females have increased in the last 20 years, and there seems to be a nascent increase in the rate of lung cancer in this group. It is difficult to differentiate cases of lung cancer arising from environmental exposure from those due to smoking. This stems from the fact that while there is a fairly large list of occupational and environmental lung carcinogens (Table 1), the majority of persons at risk are exposed to soots, tars, combustion products, and other complex mixtures that have similar chemical composition to tobacco smoke. Therefore, the specific chemical agents that cause lung cancer from smoking and those caused by most environmental exposures are likely to be the same or closely related. Moreover, the "trail" left by both these exposures in terms of disease or biomarkers are similar. The potential for interaction (potentiation, additive, and synergistic effects) between occupational and environmental exposures has made it difficult to assess the proportion of total lung cancers directly attributable to the general environment. The effect of occupational exposure within certain groups is profound; some exposed workers have been shown to be at 10 to 50 times greater risk than controls (*1,5*).

There are several important reasons why the development of biomonitoring methods is highly desirable for environmental lung

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Address correspondence to Dr. G. Talaska, Department of Environmental Health, University of Cincinnati Medical School, Cincinnati, OH 45267-0056. Telephone: (513) 558-0519. Fax: (513) 558-4397.

Abbreviations used: PAH, polycyclic aromatic hydrocarbons; B[*a*]P, benzo[*a*]pyrene; BAL, broncoalveolar lavage; MOCA, 4,4'-methylene-bis(2-chloroaniline).

Table 1. Occupational lung carcinogens.

Arsenic	Soots and tars
Asbestos	Uranium
Bis(chloromethyl)ether	Vinyl chloride
Chloromethyl ethyl ether	Beryllium
Chromates	Cadmium
Coke oven emissions	Chloroprene
Mustard gas	Lead
Nickel	Tobacco smoke

Table 2. Occupations with potential exposure to coal tar.

Asphalt workers	Boat builders
Briquette maker	Coal tar workers
Coke oven workers	Pavers
Creosoters	Electrode makers
Flue cleaners	Furnace workers
Pitch workers	Railroad track workers
Road workers	Roofers
Shinglemakers	Tile pressers
Waterproofers	Rubber workers

Table 3. Some complex mixtures containing polycyclic aromatic hydrocarbons.

Coal tar pitch	Coke oven tar
Aluminum reduction electrodes	Tobacco smoke
Asphalt and crumb rubber asphalt	Marijuana smoke
Used gasoline engine oil	Combustion products, in general (e.g., foundries)
Roofing tar	

cancer and environmental lung carcinogens. A primary responsibility of environmental health professions is to prevent environmental disease. Lung cancer advances in discrete premalignant stages including hyperplasia, metaplasia, dysplasia and carcinoma *in situ*. If biomarkers could be found that indicate earlier stages, it would be possible to use preventive measures (e.g., vitamin or nutrient therapy) to halt the progress of the disease. On the other hand, there is some doubt that secondary prevention of this type would be useful because, even with early detection as currently defined, the mortality rate from lung cancer is extremely high. In addition, there is strong evidence for the theory of "field cancerization" with lung cancer (*6*). This theory is based on the random probability of critical mutations in populations of cells in the lung exposed to carcinogens. After exposure of either longer duration or magnitude, all the cells will sustain mutagenic hits, and there is increasing probability that there will be multiple, independent tumors. To support this theory, it has been shown that the lungs of persons with primary tumors have many areas of aneuploidy and other atypical molecular and cytogenetic markers distant from the primary tumor (*6*). In addition, it has been reported that about 50% of long-term survivors of lung cancer develop new primary tumors. These data reinforce the notion that primary prevention is the major tool against lung cancer.

The major promise for biomarkers of environmental carcinogens is their potential use in primary prevention as sentinels of significant exposure and early effects of carcinogens; biomarkers can also be used to guide and monitor the efficacy of exposure intervention strategies. Figure 1 displays a model for a linear relationship between exposure and disease with several intervening stages. If biomarkers are available represent these stages, they can be exploited to characterize exposure in an increasingly selective way relative to disease. It is clear from the graph that the closer a marker to the occurrence of the disease, the better its ability to predict the disease. On the other hand, implicit in Figure 1 is the possibility that certain markers may be too closely related to disease outcome to be useful in prevention. Carcinogen biomarkers should measure an event in the disease process, making it biologically relevant to the disease. Measurement of the marker should also accommodate individual differences in exposure and susceptibility. The marker should be readily detectable and show a dose response with measurement of external dose. An important criterion for a marker in environmental health is that the marker measure either a reversible event or one that has a low probability of resulting in disease so that if interventions are used, both the level of the marker and the risk of disease will be decreased.

Although exposures to tobacco smoke are via inhalation, this is not necessarily true for all environmental PAH carcinogens. In many cases dermal absorption is a significant component of total exposure. Relying on air monitoring for exposure assessment is fraught with error if dermal exposure plays a significant role. This occurrence has best been documented for the aromatic amine-induced urinary bladder carcinogens in dye and rubber workers, but the evidence increasingly suggests that this may also be true for exposure to other carcinogens (*7*). For example, young children may be exposed via the dermal and oral routes as they crawl on contaminated surfaces and place contaminated objects in their mouths. Recent preliminary data indicated that children had higher levels of PAH metabolites in their urine than their mothers (*8*). Biological monitoring can estimate total internal dose from all routes of exposure. Since the carcinogenicity of many exposures is now recognized, personal protective equipment is often used to reduce exposure. However, there is a wide variation in the proper use of such equipment, and there is almost no use of protection by persons in the general population. By taking biological samples of the internal dose of the exposure, the efficacy of the protective devices can be measured in the exposed persons.

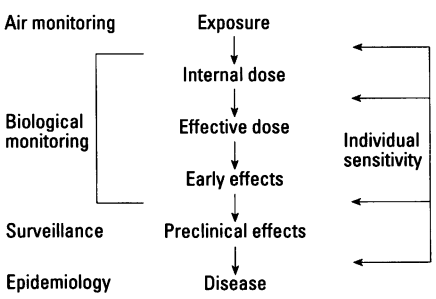

Figure 1. Model for a linear relationship between exposure and disease with intervening stages.

PAHs and Nitro-PAHs in Ambient Air and Certain Occupations

The presence of polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene (B[*a*]P), a major component of combustion-generated particulates in polluted ambient air, is well established, as are the carcinogenic and mutagenic properties of these materials. The role of nitrous compounds, specifically the nitro-PAHs, which are known to be direct mutagens, has not been investigated as thoroughly. Better information regarding atmospheric sources, diurnal and seasonal fluctuations in air levels, half-lives, and isolation and identification of the nitro-PAH compounds as related to exposure and accurate risk assessment is needed.

Nitro-PAH compounds are generated by diesel and gasoline-powered engines and appear in the exhausts of these engines (*9*). Industrial facilities such as coal-fired power plants have also been identified as emission sources (*10*). In addition to direct emission, some nitro-PAHs such as 2-nitrofluoranthene and 2-nitropyrene have been shown to be formed from the reaction of oxides of nitrogen with hydroxyl radicals and polycyclic aromatic hydrocarbons in ambient air (*11,12*). Sources of nitro-PAH generation in the indoor environment include kerosene heaters and gas and liquified petroleum gas burners (*13*).

The levels of nitro-PAHs found in the environment have been the subject of numerous reports. Levels of 1,6-dinitropyrene ranged from 0.026 pg/m^3 to 7.5 pg/m^3 in various U.S. industrial, urban, and suburban areas (*14*). Organic extracts from coastal sediments off the shore of Barcelona contained 1-nitropyrene, 6-nitrochrysene, and 6-nitrobenzo[*a*]pyrene (*15*). Some inferences regarding the importance of nitro-PAHs to the total mutagenic load of ambient atmospheres can be drawn from the current database. The direct-acting mutagenicity of the nitro-PAHs as compared to the promutagenicity of B[*a*]P and other PAHs as seen in the Ames bioassay have been well documented (*11*). Thus, certain PAHs must be transformed metabolically through a mammalian enzyme system, while nitroaromatic compounds can be reduced to the corresponding *N*-hydroxy aromatic amine by the tester strain without the addition of mammalian enzymes. Results from numerous studies indicate that extracts of polycyclic organic matter collected from cities in the United States and Japan did not need mammalian metabolic activation to be mutagenic; therefore, these urban particles must contain, in addition to promutagenic PAHs, other direct-acting mutagens. Due to their high mutagenic potency and their relative levels in the air, the impact of nitro-PAHs on the environment needs to be addressed. The mutagenic profiles of the extracts were also found to vary according to the presence of nitrogen dioxide, ozone, the amount of organic carbon, and other processes that potentially occur between ambient aerosols and reactive gases. The contribution of nitro-PAHs to the mutagenicity of airborne extracts from homes was evaluated in the Barcelona study mentioned above. Nitro-PAHs accounted for about 50% of the mutagenicity of particulate from samples collected from a home in the city. Similar tests conducted in a rural home during various particulate-generating activities such as burning charcoal, smoking tobacco, and cooking indicated that nitro-PAHs accounted for a maximum of 33% of the mutagenicity (*16*).

Metabolism of Nitro-PAH

Several of the nitro-PAH metabolic pathways have been studied. Most clearly defined are the metabolism of 6-nitrochrysene and 1-nitropyrene. Generally, nitro-PAH metabolism consists of activation by ring- or *N*-oxidation, resulting in the production of DNA adduct-forming and mutagenic intermediates; detoxification occurs by the formation of ring phenols.

6-Nitrochrysene is metabolized through two possible routes, as shown in Figure 2 (*17*). The major pathway, which has been demonstrated in mice, involves ring oxidation producing *trans*-1,2-dihydro-1,2-dihydroxy-6-aminochrysene and subsequent formation of 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-6-aminochrysene, which forms a deoxyguanosine adduct (*18*). The former reaction is catalyzed by CYP1A2 and the latter by CYP3A4 in human liver microsomes (*19*). Interestingly, the same study showed that CYP1A1 catalyzes the conversion of 6-nitrochrysene to *trans*-1,2-dihydro-1,2-dihydroxy-aminochrysene in human lung microsomes (*19*). Alternatively, 6-nitrochrysene can be reduced to the corresponding *N*-hydroxy-6-aminochrysene, which is activated to an electrophilic nitrenium ion that forms adducts at the C8 position of deoxyadenosine (18). *N*-Hydroxy-6-aminochrysene can also be reduced by human liver CYP3A4 to 6-aminochrysene, which is shunted into the major pathway through formation of 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-6-aminochrysene (*19*).

Recent studies indicate that 1-nitropyrene may follow a metabolic route similar to that of 6-nitrochrysene. While Howard et al. (*20*) have shown that 1-nitropyrene is ring-oxidized to the 6-ol and 8-ol by rabbit CYP2C3 liver microsomes, Silvers et al. (*21*) found that human liver microsome P4503A4 oxidizes 1-nitropyrene to form predominantly the 3-ol. It has been suggested that in mice these oxide intermediates are conjugated with glutathione in the liver. The conjugates are released into the bile and converted to cysteine conjugates in the upper intestinal tract. Finally, these are degraded by cysteine conjugate β-lyase of the intestinal microflora in the lower intestinal tract, and these reaction products may result in formation of a 1-nitropyrene K-region epoxide adduct (*22*). It has also been demonstrated that 1-nitropyrene can alternatively be nitroreduced, and NADPH-cytochrome C reductase has been suggested to be responsible for this conversion in the HepG2 cell line (*23*).

These data suggest that nitro-PAHs, as well as the better studied PAHs, are present in urban environments and that they make a

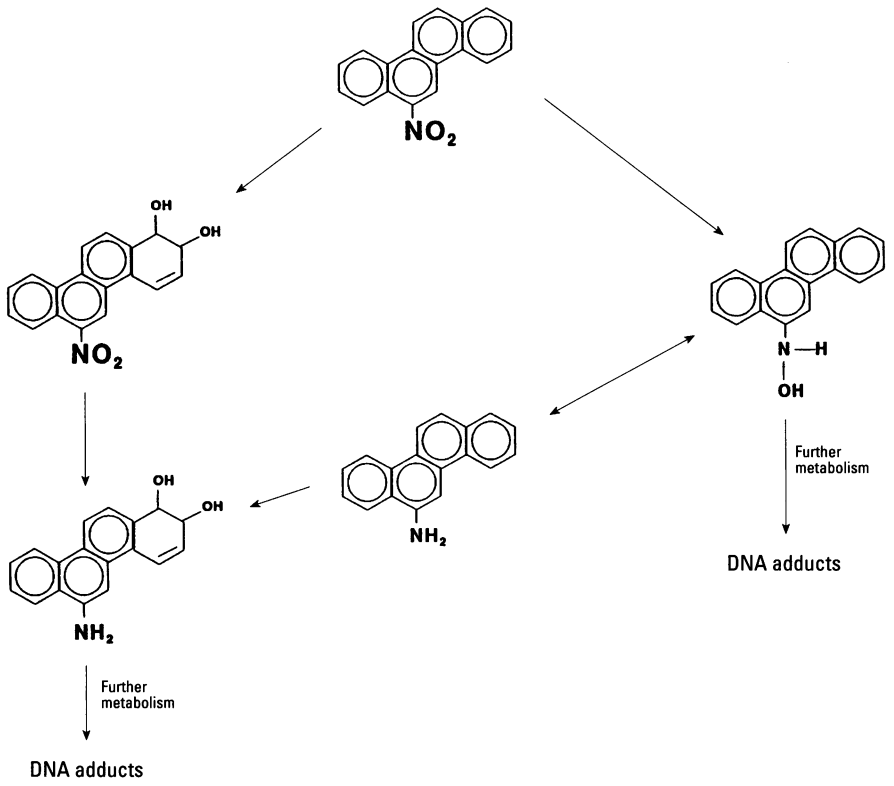

Figure 2. Metabolism of 6-nitrochrysene.

significant contribution to the genotoxicity of these environments. These compounds can reach the lung where they can be metabolically reduced or oxidized to electrophilic species capable of binding to DNA and other biological macromolecules. The extent of human exposure and the biological effects, if any, of exposure to these compounds have not yet been determined.

Biomonitoring for PAHs and Nitro-PAHs

Measurement of carcinogen–DNA adducts is an appropriate marker in the context of disease prevention. Adducts form in target tissues at elevated levels that approximately reflect the increased risk for the particular exposure. For example, adduct levels in the lungs and urinary bladder of smokers were proportional to the increased cancer risk in those organs relative to the levels in controls (*24,25*). DNA adduct levels have also been shown to respond to exposure interventions. The levels in ex-smokers were significantly lower than those in smokers when sufficient time was allowed for adducts to be cleared by cell turnover or DNA repair. Finally, although adducts are directly related to the disease process by being the ultimate mutagens, the large size of the genome, the relatively small size of the critical targets, and the fact that DNA repair occurs make it unlikely that any specific DNA adduct will result in a tumor. Thus, if exposure is reduced before critical mutations, there will be a correspondingly lower probability of disease.

Noninvasive Techniques for Monitoring DNA Adducts

Techniques to monitor carcinogen–DNA adduct levels in humans have undergone rapid development since their advent. Early studies in humans employed surgical samples from target and other tissues. As mentioned above, increased levels of carcinogen–DNA adducts were seen in lungs and urinary bladders of tobacco smokers (*24,25*). Other studies of persons with this exposure indicate that adduct levels are elevated in the oral cavity, heart, and placenta (*26,27*). Lung macrophages obtained from smokers during bronchial alveolar lavage (BAL) have not been significantly elevated (*26*). On the other hand, significant differences were noted in BAL cells when persons were exposed environmentally to smoky coal, a fuel used for heating and cooking in some areas of China, where there is a high rate of lung cancer (*28*).

Blood Lymphocytes in Monitoring Environmental Exposure

Blood lymphocytes have potential as surrogates for measurement of damage in target organs. Lymphocytes circulate throughout the body and potentially contact circulating carcinogens or their metabolites. They are easily obtained through a blood sample. These cells should integrate exposure over their lifespan because they do not divide until they are induced to do so in culture. The lifespan of different lymphocytes is variable, and this may have a profound effect on their response. For example, Savela and Hemminki (*29*) noted that there was no statistically significant difference between DNA adduct levels in crude lymphocytes of smokers compared to nonsmokers. However, when comparisons were made on different cellular fractions, it was noted that while the levels in the relatively short-lived granulocytes (which account for 40 to 75% of total lymphocytes) were not different between the groups, there was a 2.4-fold higher level of DNA adducts in the T leukocytes of the smokers. We reported a case study of an individual who was given 6-nitrochrysene as an antineoplastic agent (*17*). The adducts in the lymphocytes of this person appeared to co-chromatograph with a 6-nitrochrysene-dihydrodiol–DNA adduct standard prepared *in vitro,* suggesting that ring oxidation may be more important than nitroreduction for this compound.

As with any other surrogate tissue or marker, it is important that the relationship between the levels of response in the surrogate be compared to that in the target. Animal studies indicate that there might not be a significant relationship between DNA adducts in leukocytes and the target organs for all compounds. B[*a*]P, the compound often used to model effects for the larger family of PAHs, was shown to be the exception, as the levels of B[*a*]P–DNA adducts were correlated with the levels in target tissues (*30*). On the other hand, Van Schooten et al. (*31*) have shown that there was no correlation between the individual adduct levels in lymphocytes and surgical samples of the lung in a series of lung cancer patients. Thus, it appears difficult to generalize regarding the utility of white blood cell adduct levels as a surrogate for the levels in the target tissue. Animal and human studies may be required to empirically determine the relationship for any particular exposure.

Exfoliated Cell Techniques

Our approach to the issue of human biomonitoring has been to exploit the exfoliation of cells from lung and urinary bladder. Lung and urinary bladder are two major targets for occupational carcinogens. And, while it is difficult to obtain samples of tissues from these organs noninvasively, we reasoned that since cells from the lung and urinary bladder regularly exfoliate, it might be possible to capture these cells and use them to monitor exposure and effects within the tissue. Although estimates vary considerably, the lifespan of both urinary bladder and lung cells has been reported as about 100 days (*32,33*). We have shown recently that certain DNA adduct levels in exfoliated urothelial cells in cigarette smokers were related to reported smoking history, urinary mutagenicity, and the levels of 4-aminobiphenyl–hemoglobin adducts (*34,35*). We have also reported that the levels of carcinogen–DNA adducts are similar between bladder cancer cases and controls and are related to smoking history (*36*). We reported the results of a case study in which a worker was accidentally exposed to 4-4′-methylenebis(2-chloroaniline) (MOCA). The MOCA–DNA adduct levels in exfoliated urothelial cells of this individual were extremely high immediately after exposure, then decreased rapidly, probably as a function of the rapid loss of highly exposed cells (*37*).

We recently studied a group of workers exposed to benzidine and benzidine-based dyes (*38*). Morning urine samples were obtained from workers and controls and analyzed for excretion of benzidine metabolites, acetylation phenotype, and levels of specific benzidine–DNA adducts in exfoliated urothelial cells. We found that the major DNA adduct in the exfoliated urothelial cells of benzidine workers was *N*-(deoxyguanosin-8-yl)-*N*′-acetylbenzidine. The mean levels of this adduct were about 20 times higher in the benzidine production workers than in controls, but only about twice as high in the benzidine dye workers relative to the same comparison group. This study further supports the use of this technique to noninvasively biomonitor selected populations.

Because PAHs and nitro-PAHs reside on the particulate fraction of combustion products, it might be anticipated that the lung, not the urinary bladder, is the major target organ. In addition, the lung is capable of carrying out both the oxidative and reductive metabolic reactions required to activate these compounds. We have also performed preliminary studies with cells exfoliated from the lung and collected in sputum samples. We have seen that in a

very small sample ($n = 3$), the adducts in DNA from cells collected in sputum samples were qualitatively and quantitatively similar to the adducts in DNA from cells obtained by brush biopsy from the same individual.

Conclusion

Exposure to PAHs and nitro-PAHs in the environment may have a significant impact on certain individuals. Individuals at particular risk are those who are exposed to high levels of these compounds; those in proximity to emissions from diesel equipment, for example, as well as those who may be predisposed metabolically to production of high levels of genotoxic metabolites. Biological monitoring may likely provide more pertinent information than air sampling data because of the multiple pathways of exposure. Methods have been developed to monitor the levels of carcinogen–DNA adducts in blood lymphocytes and in exfoliated urothelial cells. These techniques may prove to be mutually exclusive or complementary and studies should be done in exposed populations to determine how adduct measurements in these tissues are related.

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